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Angiotensin type 1 receptor mediates chronic ethanol consumption-induced hypertension and vascular oxidative stress

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ABSTRACT

Objectives: We hypothesized that chronic ethanol intake enhances vascular oxidative stress and induces hypertension through renin–angiotensin system (RAS) activation.
Methods and results: Male Wistar rats were treated with ethanol (20% v/v). The increase in blood pressure induced by ethanol was prevented by losartan (10 mg/kg/day; p.o. gavage), a selective AT_1 receptor antagonist. Chronic ethanol intake increased plasma renin activity (PRA), angiotensin converting enzyme (ACE) activity, plasma angiotensin I (ANG I) and angiotensin II (ANG II) levels and serum aldosterone levels. No differences on plasma osmolality and sodium or potassium levels were detected after treatment with ethanol. Ethanol consumption did not alter ACE activity, as well as the levels of ANG I and ANG II in the rat aorta or mesenteric arterial bed (MAB). Ethanol induced systemic and vascular oxidative stress (aorta and MAB) and these effects were prevented by losartan. The decrease on plasma and vascular nitrate/nitrite (NOx) levels induced by ethanol was prevented by losartan. Ethanol intake did not alter protein expression of ACE, AT_1 or AT_2 receptors in both aorta and MAB. Aortas from ethanol-treated rats displayed decreased ERK1/2 phosphorylation and increased protein expression of SAPK/JNK. These responses were prevented by losartan. MAB from ethanol-treated rats displayed reduced phosphorylation of p38MAPK and ERK1/2 and losartan did not prevent these responses.
Conclusions: Our study provides novel evidence that chronic ethanol intake increases blood pressure, induces vascular oxidative stress and decreases nitric oxide (NO) bioavailability through AT_1-dependent mechanisms.

1. Introduction

Chronic ethanol consumption is a comorbid variable that increases the incidence of cardiovascular diseases [1,2]. In this sense, epidemiologic studies have found a positive association between high ethanol consumption and arterial hypertension [3,4]. However, although the link between ethanol consumption and arterial hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. Chronic ethanol consumption induces dose-dependent blood pressure increase, which is associated with increased vascular reactive oxygen species (ROS) generation [5,6]. Ethanol intake reduces nitric oxide (NO) bioavailability in the vasculature and this response is associated with increased arterial blood pressure [5,7,8]. Moreover, chronic ethanol consumption is associated with membrane lipid peroxidation and increased vascular NAD(P)H oxidase activity [7,9].

Chronic ethanol consumption increases plasma renin activity (PRA) and angiotensin II (ANG II) levels in humans [10–12]. Similarly, increased vascular and plasma ANG II levels were described in rats chronically treated with ethanol [6,13]. The renin–angiotensin system (RAS) is critically involved in the control of blood pressure. ANG II, the major bioactive peptide of the RAS, is produced systemically and locally within the vascular wall [14]. The peptide exerts its biological actions via two G-protein-coupled receptors, named AT_1 and AT_2 [15]. Most of the actions of ANG II including sodium retention, aldosterone secretion and vasoconstriction are mediated by AT_1 receptors [14]. The vascular system may act independently from the systemic RAS to generate ANG II [16]. The vascular ANG II generating system may be activated even when the systemic RAS is suppressed or normal. The exact function of the vascular RAS is not fully understood, but it may amplify the effects of the systemic RAS, particularly in pathological conditions, such as in hypertension [17].

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In addition to its biological effects, ANG II has been implicated in the progression and/or onset of cardiovascular diseases, including hypertension [14,15]. ANG II has been demonstrated to cause vasoconstriction partially by increasing the production of superoxide anions (O_2^-) via the activation of the enzyme NAD(P)H oxidase in the vascular wall [14]. Activation of AT_1 receptors leads to O_2^- generation, which will in turn influence downstream signaling targets of ANG II, including mitogen-activated protein kinases (MAPK) [18,19]. MAPK are a family of serine/threonine kinases, which are involved in the pathogenesis of cardiovascular dysfunctions such as vascular fibrosis and hypertension [20,21].

While there are reports describing that ethanol increases plasma ANG II levels, the role of RAS activation on ethanol-induced vascular oxidative stress and hypertension remains elusive. We hypothesized that ethanol consumption induces hypertension, ROS generation and activation of redox-sensitive signaling pathways in the vasculature through RAS-mediated mechanisms. Here, we attempted to investigate the role of RAS in chronic ethanol consumption-induced hypertension and vascular oxidative stress. A second purpose of this study was to assess the effects of chronic ethanol consumption on vascular RAS, based upon experimental evidence on the contribution of this system to vascular oxidative stress and hypertension [22,23]. Since the effects of ethanol are vessel-specific [24], in the present investigation we evaluated the effects of chronic ethanol consumption in conduit (aorta) and resistance arteries (mesenteric arteries).

2. Methods

2.1. Ethanol administration

Experiments were performed in accordance with the principles and guidelines of the animal ethics committee of the University of São Paulo — *Campus* Ribeirão Preto (#11.1.1103.53.1). Male Wistar rats, initially weighing 250–280 g (60–70 days old), were randomly divided into four groups: control, ethanol, control + losartan (10 mg/kg/day, p.o. gavage) [25,26], and ethanol + losartan. Control rats received water ad libitum, whereas rats from the ethanol group received 20% (v/v) ethanol in their drinking water [8,24,27,28]. All animals had free access to Purina Lab Chow®. To avoid a considerable loss of animals, the ethanol-treated group was submitted to a brief and gradual adaptation period. The animals received 5% ethanol in their drinking water during the first week, 10% in the second and 20% in the third week. At the end of the third week, the experimental stage was initiated and lasted for 2 weeks.

2.2. Systolic blood pressure measurements

Systolic blood pressure was measured weekly by tail-cuff plethysmography (Plethysmograph EFF306, Insight, Ribeirão Preto, Brazil). Before starting the blood pressure measurements, the rats were trained in the apparatus for three continuous days. Systolic blood pressure measurements were recorded weekly. The rats were maintained for 5–10 min in a warm chamber and three consecutive recordings (~2 min apart) were performed. Systolic blood pressure is expressed in mm Hg.

2.3. Plasma renin activity (PRA) and serum aldosterone determination

Animals were anesthetized intraperitoneally with urethane (1.25 g/kg, Sigma-Aldrich, St. Louis, MO, USA) and decapitated. Blood samples were collected in tubes containing ethylenediamine tetraacetic acid (EDTA). The samples were centrifuged (1000 ×*g*, 3 min, 4 °C) and PRA was determined with standard radioimmunoassay (RIA) techniques using a commercial RIA kit (Immunotech, Swanton, Vermont, USA). Results are expressed as ng/ml/h.

For aldosterone determination, blood samples were collected in tubes without anticoagulant. The samples were centrifuged (1000 ×*g*, 20 min, 4 °C) and aldosterone was determined using a commercial RIA kit (Immunotech SA, Marseille, France). Results are expressed as ng/dl.

2.4. Plasma ACE activity

Plasma ACE activity was determined as previously described [26]. Results are expressed as relative fluorescence unities (RFU). Tissue ACE activity was determined in the aorta and MAB. The tissues were homogenized in buffer containing zinc (10 ml of Tris buffer [150 mM–pH 8.3] and 10 μl of zinc 1 mM diluted in water). ACE enzymatic activity in aorta and MAB was determined following incubation with intramolecularly quenched synthetic specific substrate [29]. A working solution (0.45 mM) of the ACE substrate, Abz-Gly-p-nitro-Phe-Pro-OH (BACHEM, Bubendorf, Switzerland), was prepared in 150 mM Tris buffer (pH 8.3) containing 1.125 M NaCl. The assay was performed in a black flat-bottom 96-well plate with 10 μl of sample homogenate, 90 μl of Tris buffer and 200 μl of Abz-Gly-p-nitro-Phe-Pro-OH, with a final volume of 300 μl. The assay was also performed in the presence of 10 μM captopril, an ACE inhibitor. After incubation for 24 h at room temperature, the fluorescence was measured using a fluorometer (excitation wavelength: 365 nm; emission wavelength: 415 nm). Blank values were subtracted from all fluorescence values. Fluorescence resulting from ACE-specific activity was determined by subtracting values obtained in the presence of captopril from those in its absence. Results are expressed as relative fluorescence unities (RFU)/mg protein.

2.5. Plasma and tissue ANG I and ANG II determination

Blood, aorta and MAB were collected as previously described [26]. Aorta and MAB were homogenized and peptides were extracted onto a bond Elut SPE-Column (Peninsula Laboratories INC., Belmont, CA, USA) as described previously [26]. The specific anti-bodies for ANG I (#T4166) and ANG II (#T4007) were purchased from Peninsula Laboratories (San Carlos, CA, USA). The sensitivity of the RIA and coefficient of variation intra- and inter-assays were 1.2 pg/ml, 12.2 and 15.2% for ANG I and 0.39 pg/ml, 10.9 and 17.1% for ANG II. Results are expressed as pg/ml or pg/mg protein.

2.6. Plasma atrial natriuretic peptide (ANP) and vasopressin (AVP) determination

Plasma levels of ANP and AVP were measured by RIA. Details are available in the Supplementary material online.

2.7. Determination of plasma osmolality

The blood was collected in tubes containing heparin (10 μl/ml) and centrifuged (1000 ×*g*, 10 min, 4 °C). Plasma osmolality was measured in an osmometer (Digimatic model 3D2, Advanced Instruments, Norwood, MA, USA) by freezing point depression. Results are expressed as mOsm/kg H_2O.

2.8. Determination of plasma levels of sodium and potassium

Blood samples were collected in tubes without heparin to avoid contamination of samples with sodium present in anticoagulant. The tubes were kept at room temperature for 2 h and then centrifuged (1000 ×*g*, 20 min, 4 °C). Plasma sodium concentrations were determined using a flame photometer (model b262, Micronal, São Paulo, Brazil). The instrument was calibrated with a standard solution containing 140 mEq/l dilute sodium MilliQ water (1:100). Results are expressed as mEq/l.

For potassium determination, blood was collected in tubes containing coagulum activator and centrifuged (1000 ×*g*, 10 min, 4 °C). Plasma potassium was measured in a flame photometer (model b262, Micronal, São Paulo, SP, Brazil). Results are expressed as mEq/l.